ON THE ACTION OF PHOSPHATE UPON HEXOSES. II.

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Is Potassium Phosphate the Unique Catalyst by whose Action Acetol is formed from Glucose under the Experimental Conditions? In the previous work,[1] we observed that when a mixture of glucose and a slightly acidic (*p*H 7–5) concentrated solution of potassium phosphate was distilled, its volume being kept constant by adding water drop by drop, acetol was found in an appreciable quantity (ca. 5% of glucose used) in the distillate, accompanied by traces of methylglyoxal and diacetyl. We can, however, anticipate that such special catalytic action will not be limited solely to potassium phosphate.

To solve this question, similar distillation experiments at *p*H 6.2–7.0 were tried with certain other alkali salts. And it was found that a concentrated solution of sodium phosphate, potassium arsenate, or sodium sulphite, behaves like that of potassium phosphate, actually giving acetol in the distillate from glucose. On the contrary, concentrated solutions of alkali chlorides or sulphates gave no distillate showing the iodoform reaction, and the distilling mixtures turned only faintly yellow in colour even after a prolonged distillation (10 hours).

We may so far infer that there are two classes of alkali salts, one giving acetol when its neutral or slightly acidic concentrated solution is distilled with glucose, and the other not. Since the boiling temperatures all lay between 104° and 109°, this striking difference seems to be attributable simply not to the difference in the reaction temperatures but rather to that in their catalytic activities.

By the same iodometric method as described in the previous article, some determinations were made on yields of the iodine-consuming substances (estimated as acetol) from glucose in the distillation experiments with about 40% solutions of some of these catalytically active salts. It was found that the yield in the experiment with potassium arsenate was about one-fourth, and that with sodium sulphite was less than three-fifth, of that with potassium phosphate. In the previous work, it was also established that the iodine-consuming substances in the experiment with potassium phosphate are mainly (more than 60%) composed of

(1) This Bulletin, **10** (1935), 122.

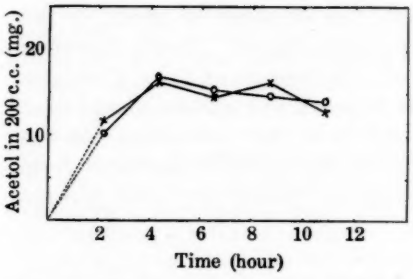
acetol. Taking these facts into consideration, it is probable that among these salts potassium phosphate is the most favourable catalyst for the production of acetol.

Influence of Iron and Copper Ions, etc. In the literature, there is abundant evidence that certain metals have some characteristic actions in the process of oxidative decomposition of carbohydrates under various experimental conditions. According to Meyerhof and Matsuoka,[2] for example, fructose is oxidized to carbon dioxide by aerial oxygen even in an acidic solution of sodium phosphate when copper or iron salt is present. Further, Bernhauer, and Tschinkel[3] have found that various sugars split into methylglyoxal when they are distilled with dilute sulphuric acid and hydrogen peroxide in the presence of ferrous sulphate. The nature of the catalytic action of these metals is not yet well known. It is, nevertheless, not impossible that these catalysts activate the sugar molecules as well as those of the oxidizing agents. There is, therefore, a possibility that iron or some other metals might play some important rôles in the characteristic non-oxidative decomposition of glucose by potassium phosphate, since our experimental materials always contained some metals as impurity, e.g., calcium, magnesium, etc., and especially iron.

To examine this possibility, the rates of appearance of the iodine-consuming substances (estimated as acetol) in the distillates from a mixture of glucose and potassium phosphate solution, either alone or along with iron or other metallic salts, were compared. The potassium phosphate used for this purpose was purified and proved to be free from any heavy metal and alkali earth. The purified glucose was, however, not quite free from a trace of ash—calcium—but it was free from iron. All of the distillations, of course, were carried out under conditions as similar as possible, and in each distillation experiment a rate-curve[4] was prepared. Fig. 1 shows the rate-curves from the experiments with potassium phosphate alone. As seen in the figure, the two curves practically coincide.

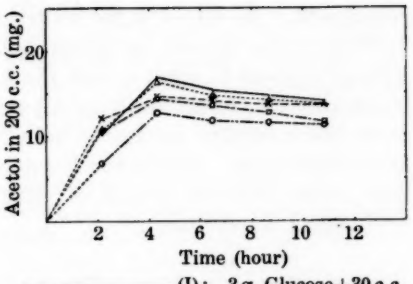
Fig. 2 contains the rate-curves for the experiments with phosphates of calcium, magnesium, zinc, and copper, respectively (in such a quantity as to make 1/100–1/300 molar solution) in addition to potassium phosphate. Fig. 2 indicates that the addition of calcium or magnesium

(2) O. Meyerhof and K. Matsuoka, *Biochem. Z.*, **150** (1924), 1.
(3) K. Bernhauer and H. Tschinkel, *Biochem. Z.*, **230** (1931), 484.
(4) See the experimental part.



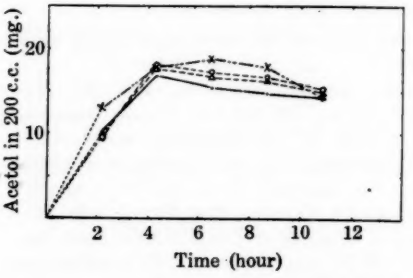
(I): 3 g. Glucose + 30 c.c. 40% K-phosphate solution, *p*H 6.2-6.4.

Fig. 1.



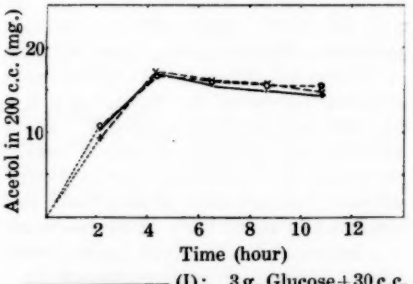
——— (I): 3 g. Glucose + 30 c.c. 40% K-phosphate solution, *p*H 6.2-6.4.
---△-------△--- (II): (I) +1/200 molar Ca-phosphate.
---×-------×--- (III): (I) +1/100 molar Mg-phosphate.
-·-□-·-·-□-·- (IV): (I) +1/300 molar Zn-phosphate.
-·-○-·-·-○-·- (V): (I) +1/100 molar Cu-phosphate.

Fig. 2.



——— (I): 3 g. Glucose + 30 c.c. 40% K-phosphate Solution, *p*H 6.2-6.4.
—·×—·· — × —· (VI): (I) +1/100 molar Ferrous sulphate.
---○----- ○--- (VII): (I) +1/200 molar Ferrous phosphate.
---△-------△--- (VIII): (I) +1/300 molar Ferrous phosphate.

Fig. 3.



——— (I): 3 g. Glucose + 30 c.c. 40% K-phosphate solution, *p*H 6.2-6.4.
---------- (IX): (I) +1/100 molar Ferric phosphate.

Fig. 4.

salt does not notably influence the reaction, whereas zinc and copper salts have a retarding action.

Fig. 3 shows the influence of addition of ferrous salts to the distilling mixture (in such a quantity as to make 1/100–1/300 molar solution). Thus ferrous sulphate or phosphate undeniably accelerates the rate of formation of the iodine-consuming substances. On the contrary, ferric phosphate showed almost no influence upon this rate (Fig. 4).

Thus, among the metal-ions studied, ferrous and cupric ions seem to have some particular effects upon the formation of iodine-consuming

substances. In all of these experiments, the presence of acetol in the distillates was proved by preparing its semicarbazone. Since, however, it is not known to what extent the iodine consumption of these distillates is actually due to acetol, it is impossible to reach any absolute conclusion from these results, as to the catalytic effect of these metal-ions on the acetol formation. But it has become obvious that acetol is produced from pure glucose by the action of pure potassium phosphate under the experimental conditions.

Experimental Part.

The distillations, and the estimations (as acetol) of the iodine-consuming substances in the distillate were carried out in the same ways as described in the previous article, excepting that a distilling flask in Pyrex glass was used this time instead of an ordinary one.

Acetol Formation by Other Salts. The reagents used in these experiments were the commercial "chemically pure" products.

Sodium phosphate. Distilling mixture: 5 g. glucose, 50 c.c. ca. 40% sol. Na-phosphate (from $Na_2HPO_4 \cdot 12H_2O$ and $NaH_2PO_4 \cdot H_2O$). *p*H 6.2–6.5; boiling temperature 106–109°. From 1000 c.c. (0.14 g. as acetol) of the distillate, about 0.1 g. acetolsemicarbazone and a trace of diacetylbissemicarbazone were actually obtained.

Potassium arsenate. Distilling mixture: 3 g. glucose, 30 c.c. ca. 40% sol. K-arsenate (prepared by boiling 40 g. KH_2AsO_4 + 5.2 g. $KHCO_3$ + 62 g. H_2O). *p*H 6.2–6.4; boiling temperature 104–105°. The presence of acetol in the distillate from this mixture was proved by means of preparation of its semicarbazone. In another experiment, the distillation was continued until the distillate gave almost no more iodoform reaction and the acetol content of the total distillate was determined, which amounted to about 1.2% of glucose used.

Sodium sulphite. About 40% solution (*p*H 6.3–6.4; boil. temp. 104–106°) of sodium sulphite gave also acetol and its yield was about 3% of glucose used. In this case, however, the acetol yield should be less than the above value, since it was determined by iodometry and the distillate evidently implied some sulphur dioxide. It is noteworthy in this experiment that the distilling mixture underwent almost no caramelisation even after a prolonged distilling process.

Twenty three per cent. solution of potassium chloride (boil. temp. 105–106°), 28% solution of sodium sulphate (boil. temp. 104°), and 23% solution of potassium sulphate (boil. temp. 103–104°) gave no distillate showing iodoform reaction. A concentrated solution of sodium chloride behaved similarly. It is, however, notable in these experiments that when a distilling flask in ordinary glass instead of Pyrex was used, some iodoform giving substance occurred in a minute quantity in the distillates.

Influence of Metal Ions. A sample of commercial potassium biphosphate (implying Cu, As, Fe, Ca, Mg, etc. as impurities) and glucose (Merck, containing Fe, Ca, etc. as impurities) were purified as follows. To a solution of the potassium biphosphate made alkaline with potassium carbonate, hydrogen sulphide was passed and

the precipitated sulphides were filtered off. The filtrate was made about *p*H 6.5 with phosphoric acid (containing a trace of iron) and then evaporated under a reduced pressure. The turbid concentrated solution was filtered through a pulp mat and further evaporated to crystallization. The crystals (KH_2PO_4) thus obtained were recrystallized twice from the solution of *p*H 6.5 (adjusted with purified potassium carbonate) and twice from their own solution. The product was free from all of the impurities mentioned above.

Glucose was twice recrystallized from glacial acetic acid, washed with alcohol containing a few drops of nitric acid, and dried at 60° under a reduced pressure. The sample thus obtained had still a trace of ash, mainly calcium, but no iron.

Using these materials, the following distillation experiments were performed in the same Pyrex flask and at the same tempo—200 c.c. distillate in ca. 130 minutes. The acetol contents (mg.) in each successive 200 c.c. distillate were plotted against time (hour) and the curves thus obtained are designated as "rate-curves". About 40% stock solution[5] of potassium phosphate (*p*H 6.2–6.4) was prepared by boiling a mixture of 340 g. KH_2PO_4, 68 g. $KHCO_3$ (purified), and 536 g. H_2O.

Distilling mixture (I): 3 g. glucose, 30 c.c. the stock solution.
The rate-curves are plotted in Fig. 1.

Distilling mixture (II): (I) + 0.046 g. $Ca_3(PO_4)_2$ (1/200 molar);
,, ,, (III): ,, + 0.074 g. $MgHPO_4 \cdot 7H_2O$ (1/100 molar);
,, ,, (IV): ,, + 0.046 g. $Zn_3(PO_4)_2 \cdot 4H_2O$ (1/300 molar);
,, ,, (V): ,, + 0.13 g. $Cu_3(PO_4)_2 \cdot 3H_2O$ (1/100 molar).

Each of these experiments was repeated twice and the mean rate-curves are plotted in Fig. 2.

Distilling mixture (VI): (I) + 0.083 g. $FeSO_4 \cdot 7H_2O$ (1/100 molar);
,, ,, (VII): ,, + 0.075 g. $Fe_3(PO_4)_2 \cdot 8H_2O$ (1/200 molar);
,, ,, (VIII): ,, + 0.05 g. ,, (1/300 molar).

The rate-curves for these experiments are plotted in Fig. 3.

Distilling mixture (IX): (I) + 0.067 g. $FePO_4 \cdot 4H_2O$ (1/100 molar).

The results are summarized in Fig. 4. In all of these experiments (I–IX) acetol-semicarbazone was obtained from the distillate and identified with an authentic specimen.

In conclusion the writers wish to thank Prof. S. Komatsu for his valuable advice and at the same time the Department of Education for a research grant.

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(5) No iron nor any other heavy metals were identified in the solution which had been boiled for 10 hours in the Pyrex flask.

DETERMINATION OF FIXED POINTS IN THE LOW TEMPERATURE WITH A HYDROGEN THERMOMETER.

By Shin'ichi AOYAMA and Eizô KANDA.

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I. Gas Thermometer. The sensitivity of the constant pressure gas thermometer is expressed by

$$S = dV/dT = 273\ V_0/T^2\ ,$$

and that of the constant volume gas thermometer by

$$S = dP/dT = P_0/273\ ,$$

where V_0 is the volume of the bulb of constant pressure gas thermometer at 0°C., and P_0 the pressure of gas in constant volume gas thermometer also at 0°C. From these expressions we see that the sensitivity of the former becomes larger with the lowering of temperatures, and the volume V_0 must, in consequence, be sufficiently large. On the other hand, if we neglect for a time the error due to the dead space, the sensitivity of the latter would be almost independent of the temperature and the volume of the bulb. The management of the former is very complicated, while the independency of sensitivity from V_0 for the latter may be very convenient, as the dimensions of vessels are limited in the studies at lower temperatures. From these standpoints the authors preferred a thermometer of constant volume to that of constant pressure and constructed one of the former by taking the references of many investigators[1] into consideration.

The thermometer is shown in Fig. 1. Bulb A and the capillary where hydrogen gas is to be filled is made of Jena 16^{III}, and the other parts are of hard glass. B is a bulb for reserving capacity, and C the portion where the level of mercury may be adjusted by means of a platinum wire sealed at its upper part. A, B, and C are connected to the capillary tubes. E_1, E_2, and E_3 are the reservoirs of mercury, the levels of which can be adjusted by changing the pressure. The temperature of the part B is kept at 0°C., and the columns C and D are surrounded by water jackets, the inner pressure of the apparatus being observed with a cathetometer.

(1) Henning, *Ann. Phys.*, **40** (1913), 635; **43** (1914), 282. Heuse and Henning, *Z. Phys.*, **23** (1924), 105; **5** (1921), 285. Heuse and Otto, *Ann. Phys.*, **9** (1931), 486. Onnes, *Comm. Phys. Lab. Leiden*, No. 97 (1906), No. 99 (1907), No. 100 (1907).

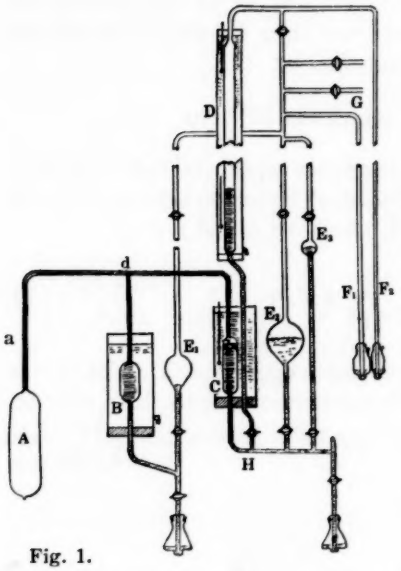

Fig. 1.

Filling of hydrogen. Hydrogen prepared through electrolysis of water is purified by palladium catalyser and also by active charcoal cooled in the liquid nitrogen. The gas is then led into bulbs A, B, and C through the tubes G, D, and H, and the process of filling and evacuating is repeated. After the fifth filling the mercury column cuts off the passage between C and D, and the pressure of hydrogen is adjusted so that it will indicate 90 mm. of mercury when A and B is kept at 0°C.

II. Determination of Temperature. For the convenience of referring in the description, the parts of the thermometer containing gas are indicated by A, B, C, a, and d, the temperatures of which being all different from one another. In Fig. 1 the part A is at a temperature of $t°$ which is to be determined, B is at 0°C., C at the temperature of water which is nearly at room temperature t_r, and a at the mean temperature between $t°$ and t_r. The effect due to the variation of the temperature of the room has been found to be very small, as the apparatus has been set in a special room tripply walled all around admitting no direct rays of the sun.

The volume of each part of the thermometer at 0°C. and under zero atmosphere has been calculated as follows: $V_A = 353.073_4$, $V_B = 19.479_2$, $V_C = 0.258_0$, $V_a = 0.041_3$, and $V_d = 0.344_1$ c.c.

In the determination of temperature the corrections due to the thermal expansion of the glass bulb and the variation of the inner pressure of hydrogen must be applied. The following are the thermal expansion coefficients[2] and the compressibility of the glass Jena 16^{III} applied for the correction.

Cubical expansion coefficient	Range of temperatures
1.992×10^{-5}	13°~−103°C.
$1.53\ \times10^{-5}$	−103°~−183°C.
0.495×10^{-5}	−183°~−253°C.

Compressibility: $\mu = 2.94\times10^{-6}$

(2) Van Agt and K. Onnes, *Comm. Phys. Lab. Leiden*, No. 176a (1925).

To give an example for calculating the correction, let us suppose that the pressure of the gas is p meter of mercury, and then the volume of the part A at $t°$ (where $t > -103°C.$) may be expressed by

$$V'_A = V_A (1+1.992\times10^{-5}\times t+2.94\times10^{-6}\times p).$$

By expressing the pressure of the thermometer when the bulb is at 0°C. or $t°$ by p_0 or p_t, and the pressure coefficient of hydrogen between 0°C. and 100°C. by β, we have the following relation between p_0 and p_t:

$$p_0\left\{V_A+V_B+\frac{V_s}{1+\beta t_s}+\frac{V_d+V'_c}{1+\beta t_r}\right\} = p_t\left\{\frac{V'_A}{1+\beta t}+V_B+\frac{V_s}{1+\beta t'_s}+\frac{V_d+V'_c}{1+\beta t_r}\right\} \quad (1).$$

The second term on the both sides of the above equation will vanish in case B is filled with mercury. The third and fourth terms on the both sides are corrections due to the dead spaces and may be expressed by $\frac{V_s}{1+\beta t_s}$ and $\frac{V'_s}{1+\beta t'_s}$.

Then we have

$$p_0\left\{V_A+\frac{V_s}{1+\beta t_s}\right\} = p_t\left\{\frac{V'_A}{1+\beta t}+\frac{V'_s}{1+\beta t'_s}\right\} \quad (2).$$

The temperature will thus be determined from the equation

$$t = \frac{1}{\beta}\left[\frac{p_t V'_A}{p_0\left\{V_A+\frac{V_s}{1+\beta t_s}\right\}-p_t\frac{V'_s}{1+\beta t'_s}}-1\right] \quad (3).$$

In the case of the present experiment the following values of p_0 and β have been adopted: $p_0 = 922.15$ mm. of mercury, $\beta = 0.0036621$[3].

III. Accuracy of Temperature. The accuracy of the standard thermometer for low temperatures is very important, because many problems have to be discussed in the temperature of absolute scale. In the following the authors attempt to discuss on the errors generally encountered in the temperature determination with the constant volume gas thermometer, taking the effect of dead spaces into consideration.

Now, if we neglect the effect of dead spaces, we have

(3) Heuse and Otto, *loc. cit.*

$$t = \frac{p - p_0}{\beta p_0} \qquad (4),$$

then
$$dt = \frac{1}{\beta}\left\{\frac{dp}{p_0} - \frac{1+\beta t}{p_0}\, dp_0 - t\,d\beta\right\}$$

and so
$$\frac{\partial t}{\partial p_0} = -\frac{1+\beta t}{\beta p_0} = -\frac{273+t}{p_0} \qquad (5),$$

$$\frac{\partial t}{\partial \beta} = -\frac{t}{\beta} = -273t \qquad (6),$$

$$\frac{\partial t}{\partial p} = \frac{1}{\beta p_0} = \frac{273}{p_0} \qquad (7).$$

If we consider the dead space, however, we get from equation (2) the following relation:

$$p_t\left\{\frac{V'_A}{V_A} + \frac{V'_s}{V_A}\,\frac{1+\beta t}{1+\beta t'_s}\right\} = p_0\left\{1 + \frac{V_s}{V_A}\,\frac{1}{1+\beta t_s}\right\}(1+\beta t)\,.$$

The coefficient $(1+\beta t)$ on the right side of the above equation is the characteristic quantity assigned to each thermometer, the value of which can be determined when A (Fig. 1) is at 0°C. If we equate this term with p'_0 and express the left side by p', we get the relation which is very analogous to equation (4).

Thus
$$p' = p_0(1+\beta t) \qquad \text{or} \qquad t = \frac{p' - p'_0}{\beta p'_0} \qquad (8)$$

where
$$p'_0 = p_0\left\{1 + \frac{V_s}{V_A}\,\frac{1}{1+\beta t_s}\right\} \qquad (9)$$

and
$$p' = p_t\left\{1 + \alpha t + \mu p + \frac{V'_s}{V_A}\,\frac{1+\beta t}{1+\beta t'_s}\right\} \qquad (10).$$

Then the maximum error of temperature will be

$$\Delta t = \frac{273+t}{p'_0} \times \Delta p'_0 + 273t \times \Delta\beta + \frac{273}{p'_0} \times \Delta p' \qquad (11).$$

The amounts of $\Delta p_0'$ and $\Delta p'$ are determined by the maximum errors of p_0, p_t, V_s/V_A, and t_s in the actual measurements, calculated by the equations (9) and (10). In order to calculate the maximum errors at 0°C. and −200°C. the above method is applied to the thermometer constructed by the authors. In the present case $V_s/V_A = 0.00182$, $\Delta p_0 = 0.05$ mm. Hg, and $\Delta t_s = 0.02°$.

(a) Errors due to p_0': $\Delta t_{p_0'} = \dfrac{273+t}{p_0'}\Delta p_0'$, $p_0 = 922.15$, $p_0' = 923.9$, and $\Delta p_0' = 0.0584$.

Accordingly, $\Delta t_{p_0'} = 0.0173$ at 0°C., and $\Delta t_{p_0'} = 0.0046$ at −200°C.

(b) Errors due to β: $\Delta t_\beta = 273 \times \Delta\beta$.

If we estimate as $\beta = 0.0000001$ ($\beta = 0.0036621$), then $t_\beta = 0$ at 0°C., and $t_\beta = 0.0055°$ at −200°C.

(c) Errors due to p': $\Delta t_{p'} = \dfrac{273}{p_0'}\Delta p'$.

The largest term of corrections for gas pressure is due to the variation of temperature of the mercury column. If we let h be the difference of the height of mercury columns observed with the cathetometer, α' the expansion coefficient of mercury and t_τ the temperature of mercury, we then have from the equation (10)

$$p' = h(1-\alpha' t_\tau)\left\{1+\alpha t+\mu p+\frac{V_s}{V_A}\frac{1+\beta t}{1+\beta t_s}\right\} .$$

As $\Delta h = 0.05$ mm. Hg, and $\Delta t_\tau = 0.02°$,

$$\Delta p' = \Delta p_h' + \Delta p_{t_\tau} + \Delta p_{t_s v_s} ,$$

$$\Delta p' = 0.0500+0.00336+0.00914 = 0.0625 \quad \text{at } 0°\text{C.},$$

and $$\Delta p' = 0.0500+0.0001 +0.00007 = 0.05017 \text{ at } -200°\text{C.}$$

Accordingly, $\Delta t_{p'} = 0.0185°$ at 0°C., and $\Delta t_{p'} = 0.0148°$ at −200°C.

Summarizing the above results, we get the following total maximum errors of the temperature: $\Delta t = 0.0358°$ at 0°C., $\Delta t = 0.0249°$ at −200°C.

From the above calculation it is clear that in the range 0° and −200°C. the amount of error decreases from 0.0358° to 0.0249° with the lowering of temperature.

IV. Comparison with the Thermodynamical Scale. The difference between the scale of the constant volume gas thermometer t_v and the thermodynamical scale of temperature t can be expressed by

$$t_v - t = p_0\{2.73(t_v-100)k_0 - 3.73t_v k_{100} + (273+t_v)k_t\}$$

where k_0, k_{100}, and k_t mean the inclination of the isothermal curve of the gas in question at 0°C., 100°C., and t°C. respectively. The difference is found to be proportional to the initial pressure p_0. In the present case it is as follows:

t	$t-t_v$	t	$t-t_v$
−225°	0.055	−100°	0.014
−200°	0.043	− 75°	0.009
−175°	0.034	− 50°	0.005
−150°	0.026	− 25°	0.003
−120°	0.019	0°	0.000

V. Determination of Fixed Points. In order to check the accuracy of the thermometer constructed, determinations of the boiling points of nitrogen and oxygen, and that of the sublimation point of carbon dioxide have been made.

(a) *Boiling point of nitrogen.* The apparatus used for the determination of the boiling point of nitrogen is shown in Fig. 2. A Dewar vessel C contains fresh liquid nitrogen, A is the bulb of the gas thermometer, B and D together act as a vapour pressure thermometer, and E and F are the portions of the apparatus where liquid nitrogen is purified through evaporation followed by condensation.

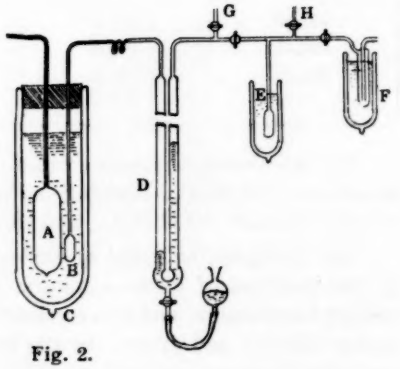

Fig. 2.

Nitrogen to be filled in B is generated by the addition of an aqueous solution of sodium nitrite dropwise to a mixture of warm saturated aqueous solution of ammonium sulphate and potassium chromate. The gas thus generated is passed through a red heated copper gauze and then purified through the process of repeated evaporation and condensation in E and F, respectively. The gas is finally condensed in B, the temperature of the bath being determined with the gas thermometer, and the vapour pressure of nitrogen in B is observed with the vavour pressure thermometer.

Using the temperature coefficient of the vapour of nitrogen ($\Delta p/\Delta t$), the values of which have been obtained from the vapour pressure thermometer scale, the normal boiling point is determined as follows:

Vapour pressure	Temperature	$\Delta p/\Delta t$	Boiling point
870.90	−194.68°C.	94.9	−195.85°C.
857.45	−194.76	94.4	−195.81
815.10	−195.24	93.1	−195.83
			mean $-195.83_3 \pm 0.03$

The mean value corresponds to −195.79°C. on the thermodynamical scale of temperature. According to Henning[4] or Onnes,[5] the fixed point is $-195.81° \pm 0.02°$ or −195.78°, being very close to that obtained in the present experiment.

(b) *Boiling point of oxygen.* Oxygen generated through the electrolysis of the aqueous solution of potassium hydroxide is freed from hydrogen with heated platinum asbestos. The gas is then condensed in a trap cooled in liquid air, and the vapour of oxygen together with any trace of hydrogen present is pumped out. The oxygen of extra purity thus prepared is introduced into B, and the normal boiling point is determined as in case (a). The following are the results obtained:

Vapour pressure	Temperature	$\Delta p/\Delta t$	Boiling point
727.60	−183.44°C.	79.1	−183.03°C.
739.45	−183.28	,,	−183.02
764.00	−182.95	,,	−183.00
			mean $-183.01_7 \pm 0.03$

By calculating the mean value to the thermodynamical scale, it corresponds to −182.98°C. Henning and Heuse[4] give the boiling point $-183.00°C. \pm 0.02°$, Onnes[5] −182.95°C., and Heuse and Otto[6] −182.962°C.

(c) *Sublimation point of carbon dioxide.* Carbon dioxide is prepared by the addition of dilute sulphuric acid to the milky aqueous mixture of sodium bicarbonate, and it is condensed in a trap cooled by a mixture of solid carbon dioxide and ether. In the bath C a mixture of solid carbon dioxide and ether is placed and well stirred. In the determination of the sublimation

(4) Henning and Stock, *Z. Phys.*, **4** (1921), 226; Henning and Heuse, *loc. cit.*
(5) "Onnes-Festschrift," II (1923).
(6) Heuse and Otto, *loc. cit.*

point, the temperature coefficient of vapour tension $\Delta p/\Delta t = 62.7$ mm. Hg is applied. The observed values are as follows:

Vapour pressure	Temperature	$\Delta p/\Delta t$	Sublimation point
762.35	−78.48°C.	62.7	−78.52°C.
758.85	−78.52	,,	−78.50
768.10	−78.41	,,	−78.53
			mean −78.51$_7$ ± 0.009

The mean value will correspond to −78.51°C. on the thermodynamical scale. Keys and Young[7] give −78.53°C., and Heuse and Otto[8] −78.48°C.

VI. Calibration of Thermocouples. The calibration of copper-constantan thermocouple is made with the hydrogen thermometer in a cryostat as shown in Fig. 3. Dewar vessel A contains another Dewar vessel B containing pentane and a copper cylinder wound with heating coil H and the space between the two vessels is filled with liquid nitrogen. Gas thermometer G and the thermocouples J and J′ are immersed in the liquid in vessel B. This vessel has a side tube through which hydrogen gas can be either introduced or evacuated to high vacuum. The cooling of vessel B is effected by the cold source of liquid nitrogen through the thermal conduction of hydrogen kept in the space between the double walls of vessel B. The control of the temperature is made possible by evacuating hydrogen and warming the liquid in the vessel gently with coil H. Small particles of aluminium also fill the space between the vessels A and B, and hence a good constancy of temperature has been able to be obtained by the aid of the thermal conductivity of aluminium even in the range of lower temperatures where liquid pentane is frozen. The constancy during the calibration has been ±0.02°.

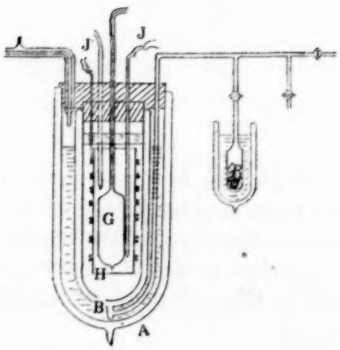

Fig. 3.

The following is the results of calibration:

Couples of Cu–Ni-Cu alloy (Ni 51.86, Cu 47.66, Mn 0.09, Fe 0.38%).

(7) *J. Math. Phys.*, **1** (1922), 241.
(8) *Loc. cit.*

Temperature	e. m. f. (m.v.)	Temperature	e. m. f. (m.v.)
−40.13°C.	1.507	−114.50°C.	3.882
−55.48	2.060	−123.75	4.118
−67.15	2.441	−135.55	4.402
−78.32	2.828	−155.41	4.877
−85.51	3.036	−172.36	5.221
−94.65	3.318	−183.50	5.456
−107.83	3.699	−194.70	5.650

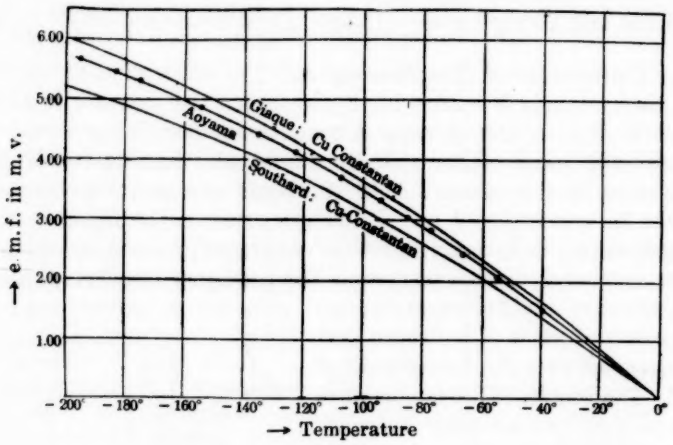

Fig. 4.

The curve in Fig. 4 shows the results given in the above table together with those of other investigators for a comparison. It may be seen that the electromotive force of the couples Cu and Ni-Cu alloy of the present authors is very close to that of copper-constantan thermocouples calibrated independently by Giaque[9] and Southard.[10]

Summary.

A hydrogen gas thermometer for the standard of the low temperature measurement was constructed wholly of glass. The accuracy of temperature obtained, ±0.036° at 0°C. and ±0.025° at −200°C., was discussed, the

(9) Giaque, Buffington, and Schulze, *J. Am. Chem. Soc.*, **49** (1927), 2343.
(10) Southard and Andrews, *J. Franklin Inst.*, **207** (1929), 323.

volume of thermometer bulb, the dead spaces, and the temperature of the room being taken into consideration. The determination of the boiling points of liquid nitrogen and liquid oxygen and that of the sublimation point of solid carbon dioxide were made with this thermometer. The calibration of copper-constantan thermocouples was also made against the thermometer at various low temperatures.

The authors express their cordial appreciation to the Saito Gratitude Foundation whose generous contributions has made it possible to equip fully their laboratory with the provisions necessary for the cryogenic researches.

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ÜBER (+)- UND (−)-BROM-THEBENON.[1]

Von Kakuji GOTO, Hiroshi OGAWA und Jun SAITO.

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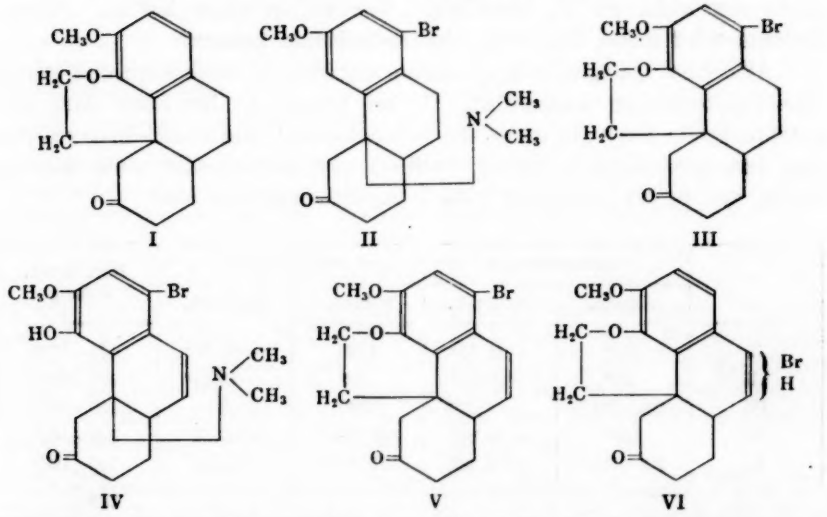

(1) XLII. Mitteilung über Sinomenin. XLI. Mitteilung, dieses Bulletin, **10** (1935), 252.

Bei der Bromierung von Thebenon (I) erhalten wir kein einheitliches Produkt; dies erklärt sich aus der Tatsache dass C_5 und C_6 ebenso leicht bromiert werden können wie C_1, besonders in Angesicht dass der freie Hydroxyl an C_4 mit Ätherringschluss geschlossen ist.[2] So haben wir mit dem Dihydro-des-N-methyl-demethoxy-dihydro-sinomenin ausgegangen und durch Bromierung zuerst 1-Brom-dihydro-des-N-methyl-demethoxy-dihydro-sinomenin (II) dargestellt. Das Jodmethylat des letzteren wird beim Kochen mit Alkali leicht ins 1-Brom-thebenon (III) übergegangen.

Um (−)-1-Brom-dehydro-thebenon (V) zu gewinnen, haben wir zuerst 1-Brom-demethoxy-dihydro-sinomenin in seine Des-N-methyl-base (IV) umgewandelt und dann das Jodmethylat der letzteren mit Alkali gekocht. Diese Umwandlung war so leicht ausführbar wie bei den nicht bromierten Derivaten.[2]

Beim vorsichtigen Bromierung von (+)-Dehydro-thebenon haben wir ein isomeres Brom-dehydro-thebenon (VI) gewonnen. In diesem Produkt, sitzt das Brom-atom vielleicht an C_9 oder C_{10}, da wir beim Bromierung[3] von Dehydro-thebenon-7-ketofurazan, in denen beide Ketongruppen durch Furazan-ringschluss abgestöpft worden ist, ebenso leicht ein nicht an C_1 bromiertes Produkt erhalten hatten. Dieses Produkt wird daher Iso-brom-dehydro-thebenon genannt.

Alle obige Umwandlungen wurden mit Dihydro-thebainon und seinen Des-N-methylbasen ausgeführt. Dabei wurde es bewiesen dass alle entsprechende Derivate denselben Schmelzpunkt und Drehungsvermögen (im entgegengesetzten Sinne) besitzen und miteinander wohl racemisieren, wie in der folgenden Tabelle zusammengefasst sind.

	Aus Sinomenin		Aus Thebain		Rac. Subst. Schmp.
	Schmp.	Sp. Dreh.	Schmp.	Sp. Dreh.	
II	192°	+ 61.60°	192°	− 62.67°	175–177°
III	70°	− 22.67°	70°	+ 23.33°	191–193°
IV	200–201°	− 8.67°	199°	+ 8°	189–192°
V	145°	−186.80°	148–150°	+187.33°	159–162°
VI	125–133°	−113.33°	127–130°	+112.67°	156–158°

(2) K. Goto, *Ann.*, **485** (1931), 247.
(3) K. Goto und M. Mitsui, dieses Bulletin, **7** (1932), 223.

Aus dieser Tabelle ersieht man dass die schrittenweise Umkehrung des Drehungs-sinnes von Dihydro-thebainon zu Thebenon, was wir schon an nicht bromierten Substanzen bemerkt haben,[2] auch hier begegnet ist. Der grosse Unterschied in Molekular-Drehung in isomeren 1-Brom-dehydro-thebenon und Iso-brom-dehydro-thebenon ist auch bemerkenswertig.

Versuche.

1. **Dihydro-des-N-methyl-1-brom-demethoxy-dihydro-sinomenin (II).** 1.1 g. Dihydro-des-N-methyl-demethoxy-dihydro-sinomenin werden in 5 c.c. Eisessig gelöst und mit 0.55 g. Brom in 10 c.c. Eisessig bei 16°C. bromiert. Das Bromhydrat der bromierten Base krystallisiert sofort aus. Schmp. 257° (heftig zersetzt). Die befreite Des-N-methyl-base wird aus Methanol umkrystallisiert. Prismen, Schmp. 192°. Diazo-reaktion nur bis 5,000 fache Verdünnung. Gefunden bei Makroanalyse: Br, 20.18. Ber. für $C_{19}H_{20}NO_3Br$ (396): Br, 20.20%. Spez. Drehung: 0.1731 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = +1.07°$; $[\alpha]_D^{17} = +61.60°$.

Jodmethylat. Bildet beim Mischen und Stehenlassen beider Komponente in Methanol. Schmp. 273° Gef. bei Makroanalyse: J, 23.62 Ber. für $C_{20}H_{20}NO_3BrJ$: J, 23.61%

Dihydro-des-N-methyl-1-brom-dihydro-thebainon (II). Darstellung wie bei (+)-Substanz aus Sinomenin. Das Bromhydrat zersetzt auch bei 257°. Schmp. von freier Base 192°. Gefunden bei Mikroanalyse: N, 3.50. Ber. für $C_{19}H_{20}NO_3$ Br (396): N, 3.54%. Spez. Drehung: 0.15 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = -0.94°$; $[\alpha]_D^{17} = -62.67°$.

Das Jodmethylat schmilzt bei 273°.

***d, l*-Dihydro-des-N-methyl-1-brom-dihydro-thebainon.** Je 0.15 g. (+)- und (−)-Substanz wurden in methanolischer Lösung vereinigt. Daraus wurde 0.2 g. racemischer Substanz gesammelt. Schlanke Prismen; Schmp. 175–177°, $\alpha = \pm 0$.

2. **(−)-1-Brom-thebenon (III) aus Sinomenin.** Das Jodmethylat, welches aus 1.5 g. obiger Des-N-methyl-base gebildet wurden, werde mit 10 c.c. 25 proz. Kalilauge 20 Min. lang gekocht. Mit der Entwickelung des Amingeruchs, werden Öltröpfen über das Wasser gebildet. Nach Verdünnen mit 3 facher Menge Wasser, wird die Lösung mit CO_2 übergesättigt und der Niederschlag aus Methanol umkrystallisiert Schmp. 70°. Gefunden bei Makroanalyse: C, 57.87; H, 5.41; Br, 22.43. Ber. für $C_{17}H_{19}O_3Br$ (351): C, 58.12; H, 5.41; Br, 22.79%. Spez. Drehung: 0.15 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = -0.34°$, $[\alpha]_D^{17} = -22.67°$.

(+)-1-Brom-thebenon (III) aus Thebain. Darstellung wie oben. Schmp. 70°. Ausbeute ca. 70%. Gefunden bei Mikroanalyse: Br, 22.76. Ber. für $C_{17}H_{19}O_3Br$ (351): Br, 22.79%. Spez. Drehung: 0.15 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = +0.35$, $[\alpha]_D^{17} = +23.33°$.

d, l-1-Brom-thebenon. Zur Racemisierung, wurden je 0.15 g. (−)- und (+)-1-Brom-thebenon in Methanol vereinigt. Daraus krystallisierte die racemische Substanz in viereckigen Blättern. 0.25 g. wurde gesammelt. Schmp. 191°–193°, $\alpha = \pm 0$.

3. Des-N-methyl-1-brom-demethoxy-dihydro-sinomenin (IV). 3 g. 1-Brom-demethoxy-dihydro-sinomeninjodmethylat werden mit 15 c.c. 15 proz. Natronlauge 12 Min. gekocht. Nach Zusatz von 8 c.c. Wasser wird die Lösung mit CO_2 gesättigt. Der ausfallende Niederschlag wird gesammelt, in Methanol gelöst und mit 1–1.5 fachen Menge Wasser gefällt. Prismen, Schmp. 200–201°. Ausbeute 1.5 g. Gefunden bei Makroanalyse: C, 57.79; H, 6.16 Ber. für $C_{19}H_{21}NO_3Br$ (394): C, 57.87; H, 6.09%. Spez. Drehung: 0.15 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = -0.13°$. $[\alpha]_D^{17} = -8.67°$.

Jodmethylat. Entsteht beim Mischen beider Komponente. Zersp. 243°. Gefunden bei Makroanalyse: J, 23.76. Ber. für $C_{20}H_{27}NO_3BrJ$ (538): J, 23.61%.

Des-N-methyl-1-brom-dihydro-thebainon (IV). Darstellung wie bei (−)-Substanz aus Sinomenin. Schmp. 199°. Gef. bei Mikroanalyse: N, 3.55. Ber. für $C_{19}H_{24}NO_3Br$ (394): N, 3.55%. Spez. Drehung: 0.1 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = +0.08°$, $[\alpha]_D^{17} = +8.00°$.

Jodmethylat. Schmp. 243° (aus Wasser).

d, l-Des-N-methyl-1-brom-dihydro-thebainon. Je 0.1 g. (−)- und (+)-Substanz wurde in Athanol gelöst und vereinigt. Beim Kühlen mit Eis krystallisiert die racemische Substanz. Schmp. 189–192°, $\alpha = \pm 0$.

4. (−)-1-Brom-dehydro-thebenon (V) aus Sinomenin. 2.5 g. des Jodmethylates der obigen Des-N-methyl-base wurden in 30 c.c. 11 proz. Natronlauge 40 Min. gekocht. Nach Ansäuern mit Salzsäure wurde 1-Brom-dehydro-thebenon in Chloroform aufgenommen. Beim Verdünsten des Chloroforms krystallisierte das Produkt in Prismen aus. Schmp. 145°. Gef. bei Makroanalyse: C, 58.57; H, 4.94; Br, 22.69. Ber. für $C_{17}H_{17}O_3Br$ (349): C, 58.45; H, 4.87; Br, 22.92%. Spez. Drehung: 0.1424 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = -2.66°$, $[\alpha]_D^{17} = -186.80°$.

(+)-1-Brom-dehydro-thebenon (V) aus Thebain. Darstellung wie bei (−)-Substanz aus Sinomenin. Schmp. 148–150°. Gef. bei Mikroanalyse: Br, 23.07. Ber. für $C_{17}H_{17}O_3Br$ (349): Br, 22.92%. Spez. Drehung: 0.15 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = +2.81°$, $[\alpha]_D^{17} = +187.33°$.

d, l-1-Brom-dehydro-thebenon. Je 0.15 g. (−)- und (+)-Substanz werden in Methanol vereinigt. Daraus wurde 0.2 g. racemischer Substanz gesammelt. Schmp. 159–162°, $\alpha = \pm 0$.

5. (−)-Iso-brom-dehydro-thebenon (VI) aus Sinomenin. 1.8 g. (−)-Dehydro-thebenon, in 10 c.c. Eisessig gelöst, werden mit 1.1 g. Br in 5 c.c. Eisessig unter Eiskühlung bromiert. Das Produkt wird mit Wasser gefällt und aus Aceton umkrystallisiert. Schmp. 125–133°. Ausbeute ca. 1 g. Gef. bei Makroanalyse: C, 58.25; H, 5.06; Br, 22.11. Ber. für $C_{17}H_{17}O_3Br$ (349): C, 58.45; H, 4.87; Br, 22.92%. Spez. Drehung: 0.15 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = -1.70°$, $[\alpha]_D^{17} = -113.33°$

(+)-Iso-brom-dehydro-thebenon (VI) aus Thebain. Diese Substanz wurde wie bei (−)-Substanz aus Sinomenin dargestellt. Schmp. 127–130°C. Spez. Drehung: 0.15 g. Subst. in 10 c.c. Chloroform, 1 dm. Rohr, $\alpha = +1.69$, $[\alpha]_D^{17} = +112.67°$.

***d, l*-Iso-brom-dehydro-thebenon.** Aus dem Gemisch von je 0.15 g. (−)- und (+)-Iso-brom-dehydro-thebenon, gelöst in Methanol, wurde 0.25 g. racemische Substanz gesammelt. Prismen von Schmp. 156–158°, $\alpha = \pm 0$.

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SORPTION OF GAS BY MINERAL. V.

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In this paper the experimental results obtained with the following minerals are described: mordenite, thomsonite, scolecite, epistilbite and phillipsite. The sorptions of ammonia and carbon dioxide by these minerals have been measured. The procedure of measurements have already been described in the preceding reports.(1) In the present report the results of the measurements with zeolite minerals from foreign countries are given, while in the former reports the Japanese minerals have only been tested.

Mordenite. The locality of mordenite described in this report is Custer County, Idaho, U.S.A. It is a white soft matter of fibrous structure. The results of measurements are given in Tables 1, 2 and 3. The mineral was dehydrated by heating at 350°C. (Table 1) or 200°C. (Table 2 and Table 3) under evacuation. The pressures of the gases were kept at 760 mm. throughout the experiments.

The amounts of ammonia and carbon dioxide sorbed by this sample of mordenite is thus inferior to that which has been described in the former report.(2) The present sample sorbs ammonia with extreme rapidity, about 90 per cent. of the total amount being sorbed during the

(1) Sameshima, this Bulletin, **2** (1927), 2; **4** (1929), 96. Sameshima and Hemmi, *ibid.*, **9** (1934), 27.

(2) Sameshima and Hemmi, this Bulletin, **9** (1934), 32.

Table 1.

Sorption of Ammonia by Mordenite.

Mode of dehydration: Heating at 350°C. for 30 minutes under evacuation.
Loss of weight by dehydration: 4.89%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of mordenite
0.5	39.47
1	41.94
5	44.13
60	45.27
1440	45.94
1800	46.32

Table 2.

Sorption of Ammonia by Mordenite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.
Loss of weight by dehydration: 4.75%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of mordenite
0.5	33.62
1	35.81
5	38.20
60	40.20
1620	41.15

Table 3.

Sorption of Carbon Dioxide by Mordenite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.
Loss of weight by dehydration: 4.41%.

Time (min.)	Vol. (c.c.) of CO_2 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of mordenite
1	1.68
5	3.97
30	9.27
60	11.82
120	14.09
480	17.22
1440	18.18
3000	18.37

first few minutes. The behaviour is quite the same with that of chabazite,[3] silica gel,[4] and charcoal,[5] and differs from other zeolite minerals.[6] This fact shows that the dewatered mordenite does not form a compound with ammonia but only sorbs it into the cavities among the molecular gratings of the crystal. The dewatered mordenite forms a molecular sieve, and the sorption by such a matter may be classified as "the sorption of chabazite type."

Thomsonite. The composition of thomsonite is said to be $2[(Na_2Ca)Al_2Si_2O_8]\cdot 5H_2O$. The mineral from Table Mountains, Golden, Colorado, U.S.A., has been used. It is an aggregate of radial groups of white fibrous crystals. The experimental results are given in Tables 4 and 5.

(3) Sameshima, *ibid.*, **4** (1929), 100.
(4) Sameshima, *ibid.*, **7** (1932), 133.
(5) Sameshima, *ibid.*, **5** (1930), 173.
(6) Sameshima, *ibid.*, **4** (1929), 97; **5** (1930), 304. Sameshima and Hemmi, *ibid.*, **9** (1934), 27.

Table 4.

Sorption of Ammonia by Thomsonite.

Mode of dehydration: Heating at 350°C. for 1 hour under evacuation.
Loss of weight by dehydration: 11.68%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of thomsonite
0.5	0.60
1	0.80
120	0.92
1440	1.32
1800	1.46

Table 5.

Sorption of Ammonia by Thomsonite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.
Loss of weight by dehydration: 4.27%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of thomsonite
0.5	1.07
10	2.06
60	3.54
180	5.10
480	7.27
1440	10.66
2880	13.44
15 days	23.84

These tables show that the thomsonite dehydrated at 200°C. sorbs ammonia in a considerable amount, while that dehydrated at 350°C. sorbs but a small amount.

One gram of thomsonite, after dehydrated by heating to 200°C. for 2.5 hours under evacuation, sorbs 0.27 c.c. of carbon dioxide (N.T.P.) at 25°C. and 1 atmospheric pressure of gas during 4260 minutes. Thus the carbon dioxide is not practically sorbed by the dehydrated thomsonite.

It is probable that ammonia combines chemically with the partially dehydrated thomsonite. The chemical analysis of the thomsonite from Table Mountains, Golden, Colorado, U.S.A., being the same locality with our sample, shows that the water content is 12.7% in average.[7] The weight loss of our sample on heating at 350°C. amounts to 11.68%, so the dehydrated sample at this temperature is almost anhydrous and this sample has no combining power to ammonia. The mineral dehydrated at 200°C. lost water of only 4.2%, and this partially dehydrated sample has the ability of combining with ammonia. The dehydration of thomsonite at various temperatures was studied by Zambonini, whose observation agrees well with our present results.[8]

(7) Doelter, "Handbuch der Mineralchemie", Vol. II, 3rd Part (1921), 19.
(8) Doelter, *ibid.*, p. 24.

Scolecite. The mineral from Taigerhorn, Iceland, has been used. It is a big transparent colourless prismatic crystal. The composition of this mineral is said to be $CaAl_2Si_3O_{10}\cdot 3H_2O$. The result of measurement is given in Table 6.

Table 6.

Sorption of Ammonia by Scolecite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.

Loss of weight by dehydration: 4.99%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of scolecite
1	2.00
5	3.90
30	8.31
60	11.50
120	16.87
180	21.91
300	30.23
1440	55.26
2880	58.51
3060	58.64

The sample dehydrated by heating at 200°C. for 2.5 hours under evacuation has been tested for the sorption of carbon dioxide. One gram of the mineral sorbed only 0.13 c.c. of carbon dioxide at 25°C. and 1 atmospheric pressure during 1680 minutes. Thus the mineral sorbs ammonia but no carbon dioxide.

It is probable that ammonia combines with the partially dehydrated sample of scolecite. The loss of water in the process of dehydration of the present sample amounts to 4.99%. On the other hand, the loss should be 4.59% or 57.1 c.c. of water vapour at normal conditions from one gram of the mineral by the calculation under the assumption that one mol of water evaporates from one mol of mineral. The present sample of the ammoniacal scolecite, therefore, corresponds to the composition $CaAl_2Si_3O_{10}\cdot 2H_2O\cdot NH_3$.

Epistilbite. The locality of the mineral used is Giebelbach near Fiesch, Valais, Switzerland. It is colourless transparent crystals of a few millimeter size. The composition is said to be $H_4CaAl_2(SiO_3)_6\cdot 3H_2O$.[9] The result of the sorption experiment of ammonia is given in Table 7.

The sorption of ammonia does not yet arrive at saturation point and proceeds slowly. Sorption of 37 c.c. (N.T.P.) of ammonia by one gram of epistilbite corresponds to one mol of ammonia for one mol of

(9) Doelter, *ibid.*, p. 201.

the mineral, assuming the formula described above. Thus the ammoniacal epistilbite has the composition $H_4CaAl_2(SiO_3)_6 \cdot NH_3$.

The mineral sorbs carbon dioxide only in a small amount. One gram of the mineral, after being dehydrated by heating at 200°C. for 2.5 hours, sorbs 0.55 c.c. of CO_2 gas (N.T.P.) at 25°C. during 2820 minutes. So the sorption of ammonia by epistilbite may be considered as the chemical combination between these two substances.

Table 7.

Sorption of Ammonia by Epistilbite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.
Loss of weight by dehydration: 9.26%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of epistilbite
1	0.59
5	1.36
60	3.72
300	7.56
1440	16.49
2880	20.83
5760	29.08
9 60	36.12

Table 8.

Sorption of Ammonia by Phillipsite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.
Loss of weight by dehydration: 13.14%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of phillipsite
5	2.81
30	10.67
60	19.96
120	27.61
240	32.89
1440	44.67
7200	54.47
14460	58.82

Phillipsite. The locality of the mineral is Capo di Bove, Rome, Italy. It is a cluster of spherites of small translucent crystals. The composition may be $(K_2Ca)Al_2Si_6O_{16} \cdot 6H_2O$. The sorption of ammonia by this mineral has been measured and the result is given in Table 8.

From the result given in this table, the composition of the ammoniacal phillipsite may be $(K_2Ca)Al_2Si_6O_{16} \cdot 2NH_3$.

The sorption of carbon dioxide was measured. One gram of phillipsite, after being dehydrated by heating at 200°C. for 2.5 hours under evacuation, sorbed 0.59 c.c. of CO_2 (N.T.P.) at 25°C. and 1 atmospheric pressure of the gas during 1440 minutes. Thus the mineral sorbs a considerable amount of ammonia but not carbon dioxide, so the sorption of ammonia may be considered as a chemical combination.

Summary.

(1) The sorptions of ammonia and carbon dioxide under one atmospheric pressure at 25°C. by the dehydrated samples of mordenite, thomsonite, scolecite, epistilbite, and phillipsite have been measured.

(2) Mordenite sorbs either ammonia or carbon dioxide in a considerable amount. This is a sorbent of the chabazite type.

(3) Thomsonite, scolecite, epistilbite, and phillipsite sorb ammonia in a large amount but not carbon dioxide. These minerals combine chemically with ammonia and form definite compounds.

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SORPTION OF GAS BY MINERAL. VI.

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The present report is a continuation of the preceding one. The sorptions of ammonia and carbon dioxide by sepiolite, talc, serpentine, asbestos, acid clay, bentonite, and vivianite are described in this paper.

Sepiolite. The sepiolite from Eskihi-Sher, Asia Minor, has been used. This is a light, porous, opaque mass of white colour. The composition of sepiolite is $H_4Mg_2Si_3O_{10}$. The results of the measurements are given in Tables 1, 2, 3, and 4.

The sepiolite sorbs either ammonia or carbon dioxide in a considerable amount. The sorption of gas by this substance resembles that by the silica gel or charcoal, and may be classified as "the sorption of silica gel type".

Talc. The mineral from Kaijoken, Mukden, Manchukuo, has been used. It is a translucent white mass of greasy lustre and the density is 2.86. The composition of talc is $H_2Mg_3Si_4O_{12}$. The weight loss on heating at 200°C. for 2.5 hours under evacuation was only 0.03%. The dried substance thus obtained sorbed 0.22 c.c. (N.T.P.) of ammonia and 0.02 c.c.

Table 1.

Sorption of Ammonia by Sepiolite.

Mode of dehydration: Heating at 350°C. for 30 minutes under evacuation.
Loss of weight by dehydration: 20.68%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of sepiolite
1	60.1
5	81.8
30	92.5
60	96.1
300	103.0
1500	110.8

Table 2.

Sorption of Ammonia by Sepiolite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.
Loss of weight by dehydration: 17.45%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of sepiolite
1	102.9
5	123.1
30	124.9
360	125.7
5760	127.0

Table 3.

Sorption of Carbon Dioxide by Sepiolite.

Mode of dehydration: Heating at 350°C. for 30 minutes under evacuation.
Loss of weight by dehydration: 21.34%.

Time (min.)	Vol. (c c.) of CO_2 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of sepiolite
1	14.4
30	15.6
300	16.6

Table 4.

Sorption of Carbon Dioxide by Sepiolite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.
Loss of weight by dehydration: 17.24%.

Time (min.)	Vol. (c.c.) of CO_2 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of sepiolite
1	22.8
5	24.7
30	24.9
1330	25.2

(N.T.P.) of carbon dioxide at 25°C. and 1 atmospheric pressure of gases. The present sample of talc sorbs, therefore, neither ammonia nor carbon dioxide.

Serpentine. The locality of the mineral used is Chichibu, Saitama Prefecture, Japan. It is a greenish mass and the density is 2.84. The composition of serpentine is $H_4Mg_3Si_2O_9$. The amount of sorption of ammonia is given in Table 5.

The sample dehydrated in the manner given above has been tested for carbon dioxide. It sorbed only 0.32 c.c. (N.T.P.) of carbon dioxide during 300 minutes. Thus, the serpentine sorbs a small amount of ammonia but not carbon dioxide.

Asbestos. The asbestos of long fibres has been used in this experiment, the locality, however, is not known. The experimental results are given in Table 6.

Table 5.

Sorption of Ammonia by Serpentine.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.

Loss of weight by dehydration: 0.41%.

Time (min.)	Vol. (c.c.) of NH_3 (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of serpentine
1	1.32
5	1.84
30	2.34
1500	2.85

Table 6.

Sorptions of Gases by Asbestos.

Mode of dehydration: Heating at 200°C. for 30 minutes under evacuation.

Loss of weight by dehydration: 3.1%.

Gas	Time (min.)	Pressure of gas (mm.)	Vol. (c.c.) of gas (N.T.P.) sorbed at 25°C. by 1 g. of asbestos
NH_3	1	754.4	9.51
	5	754.4	10.55
	30	754.5	11.03
	1230	760.5	11.64
	2890	760.3	11.80
CO_2	1	760.1	1.30
	60	759.4	1.46
	2900	766.1	1.70

Table 7.

Sorptions of Gases by Acid Clay.

Mode of dehydration: Heating at 200°C. for 1 hour under evacuation.

Loss of weight by dehydration: 20.59%.

Gas	Time (min.)	Pressure of gas (mm.)	Vol. (c.c.) of gas (N.T.P.) sorbed at 25°C. by 1 g. of acid clay
NH_3	1	769.3	34.37
	10	769.3	68.05
	30	769.3	72.70
	60	769.2	73.49
	1450	760.8	76.28
CO_2	1	765.4	2.81
	10	765.4	2.97
	160	765.1	3.02
	1620	762.0	3.04

Acid Clay. The sample from Itoigawa, Niigata Prefecture, Japan, has been used. It is a mass of yellowish white colour. The results of measurements are shown in Table 7.

Thus acid clay sorbs a great amount of ammonia but only a small amount of carbon dioxide. It is, therefore, probable that the ammonia combines with the dewatered sample of acid clay, and compounds are formed between them.

Bentonite. The bentonite from Rock River, Wyoming, U.S.A., has been used in the present experiment. It is a greenish yellow mass. The Japanese bentonite was already tested and described in one of the former reports.[1] The results of the sorption measurements of the American bentonite are shown in Table 8.

It seems that the present sample of bentonite shows the same behaviour toward ammonia and carbon dioxide with the Japanese acid clay. It may combine chemically with ammonia. The Japanese bentonite, when elutriated or dialysed, sorbs many kinds of gases as shown in the former paper.

Vivianite. The mineral from Ashio Mine, Tochigi Prefecture, Japan, has

Table 8.

Sorptions of Gases by Bentonite.

Mode of dehydration: Heating at 200°C. for 2.5 hours under evacuation.
Loss of weight by dehydration: 7.35%.

Gas	Time (min.)	Vol. (c.c.) of gas (N.T.P.) sorbed at 25°C. and 1 atm. press. by 1 g. of bentonite
NH_3	1	32.5
	5	41.8
	30	43.6
	360	45.4
	1440	46.1
CO_2	5	0.6
	480	1.0

Table 9.

Sorptions of Gases by Vivianite.

Mode of dehydration: Heating at 200°C. for 30 minutes under evacuation.
Loss of weight by dehydration: 23.66%.

Gas	Time (min.)	Pressure of gas (mm.)	Vol. (c.c.) of gas (N.T.P.) sorbed at 25°C. by 1 g. of vivianite
NH_3	2	766.4	2.46
	60	766.2	2.94
	3210	757.3	3.52
CO_2	5	761.9	0.33
	330	761.9	1.29
	2880	765.5	1.29

(1) Sameshima and Hemmi, this Bulletin, **9** (1934), 38.

been used. It is a transparent crystal of dark blue colour and vitreous lustre. The composition of vivianite is $Fe_3P_2O_8 \cdot 8H_2O$, from which the water content is calculated to be 28.7%. On heating at 200°C. the crystal takes an opaque black colour. The results are given in Table 9.

Summary.

(1) The sorptions of ammonia and carbon dioxide under one atmospheric pressure at 25°C. by the dehydrated samples of sepiolite, talc, serpentine, asbestos, acid clay, bentonite, and vivianite have been measured.

(2) Sepiolite sorbs either ammonia or carbon dioxide in a considerable amount. It is already known that this mineral sorbs various kinds of gases and that this is a sorbent of silica gel type.

(3) Acid clay and bentonite sorb ammonia but no carbon dioxide. Asbestos sorbs some amount of ammonia.

(4) Talc, serpentine, and vivianite sorb neither ammonia nor carbon dioxide.

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INFLUENCE OF CALCIUM ON CARBOHYDRATE METABOLISM.

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Extensive studies on the calcium metabolism of body, particularly from the point of view of explaining the cause of ricket, tetany, and other diseases have been made. But so far as the author knows very little study has been made on the influence of calcium on carbohydrate metabolism regarding diabetes. The present investigation is undertaken on this point with a connection with diabetes.

It is known that a normal adult needs more than 0.5 g. of calcium per day. Pregnant women and nursing mothers require a greater amount. It is believed that an average daily intake of calcium per capita exceeds

0.5 g. However only a small amount of calcium thus taken in is absorbed through the upper intestine while the rest is excreted mostly in the feces and partly in the urine as its salts.[1] The absorption depends upon its form in the food, or the degree of its solubility of the state.

Acid-base Balance. The balance of calcium content in the blood serum is ordinarily disturbed little in mild diabetes, but in the severe case with ketosis as well as in severe malnutrition, the amount of calcium in the serum is slightly lowered.[2] In these conditions the calcium deficiencies in the blood serum may be associated with low serum proteins. The most interesting symptom in diabetes is the acidosis which is commonly developed as a result of retention of ketonic acid, and is formed by the incomplete combustion of fats. Accordingly the CO_2 content in the plasma is lowered from 45–55 to somewhere 35–45 volume per cent.[3] with the fall of the *p*H before the symptoms appear (Table 1). In severe cases the whole electrolyte in the blood is strongly affected,[3][4] for example in the terminal condition of the diabetes the *p*H in the plasma falls below 7.1 and, the CO_2 content likewise becomes somewhere below 15 volume per cent. In addition to the fall of the *p*H and CO_2 content in the plasma there may also be a decrease in the content of Cl while the glycemia and glycosuria increase with an increase of the blood diastatic activity (Table 1).

Effect of Insulin Injection. After the injection of insulin, the ketosis and glycemia may be reduced with a transitory increase of the serum calcium[5] and the blood lactic acid,[6] while inorganic phosphorus in the serum decreases[7] either directly or indirectly. Accordingly the blood bicarbonate may likewise rise in this instance. This opposite effect is observed after adrenalin injection.[8]

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(1) T. F. Zucker, *Proc. Soc. Exp. Biol. Med.*, **18** (1921), 272.

(2) W. H. Jansen, *D. Arch klin. Med.*, **144** (1924), 14.

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(5) J. C. Brougher, *Am. J. Physiol.*, **80** (1927), 411.

(6) C. H. Best and J. H. Ridout, *J. Biol. Chem.*, **63** (1925), 197; C. F. Cori, *ibid.*, **63** (1925), 253.

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(8) J. P. Peters and H. R. Geyelin, *J. Biol. Chem.*, **31** (1917), 471.

Insulin Substitutes and their Actions. If insulin is taken by mouth there is little influence on the fall of glucose in the blood and urine. For this reason, recently various substitutes for insulin, which can be taken by mouth, have been proposed and tried. Among these an extract of huckleberry leaves[9] and guanidine derivatives[10][11] may be mentioned. Allen claims that the extract of huckleberry leaves reduces both hyperglycemia and glycosuria without any toxic action. Guanidine derivatives also have a distinct reducing effect on the hyperglycemia and glycosuria with an increase of the calcium in the serum,[12] but they have deleterious effect upon the livers and kidneys.[10] Both hydrazine derivatives[13] and sodium selenite[14] have actions similar to guanidine compounds, but the former is known to be injurious to the livers.

It is presumed that all these substitutes which are claimed by some to have therapeutic actions on diabetes mellitus, have no specific actions upon the disease, but they elevate the calcium content of the blood serum, and thereby both hyperglycemia and glycosuria may be reduced, either by the stimulation of calcium metabolism or by the decalcification of calcium stored in the body. If so, the parathyroid gland might play an important rôle on diabetes, since it controls the calcium metabolism of the body. The extract of parathyroid gland is effective on the metabolism, either when given by mouth or by intravenous injection, but an over-dose of the extract causes a negative calcium balance[15]. Therefore, the calcium for the metabolism must be supplied by intake of sufficient calcium in the form of food or of calcium compound. The commonest food to supply calcium is milk which contains about 1.2 g. per liter.

Food as a Cause of Diabetes. It is doubtful that all diabetes is due to disease of pancreas (the Islands of Langerhan) since there are no definite evidences given by either chemical or pathologic studies of the

(9) F. M. Allen, *Am. J. Physiol.* (*Soc. Proc.*), **81** (1927), 462.

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(13) S. Izume and H. B. Lewis, *J. Biol. Chem.*, **71** (1926), 51; H. B. Lewis and S. Izume, *ibid.*, **71** (1926), 33.

(14) V. E. Levine and R. A. Flaherty, *Proc. Soc. Exp. Biol. Med.*, **24** (1926), 251.

(15) I. Greenwald and J. Gross, *J. Biol. Chem.*, **68** (1926), 325; S. H. Liu, *J. Clin. Invest.*, **5** (1928), 259.

gland. It seems that dietary in-balance is one of the most important factors causing diabetic acidosis. Whether the food has an acidifying or alkalinizing effect depends upon the amount of protein and unoxidizable acid or upon the amount of alkaline elements in the food.[16] Meat and other foods consisting largely of protein have acidifying effects. It is shown by Hasselbalch[17] that a change from a high carbohydrate to a high protein diet causes the fall of CO_2 capacity of blood about three per cent. by volume. Therefore, it seems that the high protein diet rather than high carbohydrate is responsible for a cause of diabetes.[18] If so, there might exist an important relation between the calcium, protein, and the carbohydrate in food, which prevents the causing of diabetes.

The author is interested only in the calcium compounds, which could increase the serum calcium by oral administration without accompanying acidosis or other toxic action, in order to carry out the present experiment.

Effect of Calcium Salts on the Serum. Inorganic calcium compounds such as $CaCl_2$[19] and $CaSO_4$ cause acidosis by selective excretion but not selective absorption. However, oral administration of calcium lactate does not cause acidosis because of its organic radical, and when absorbed, it is burned in the organism. After an oral administration of 5 g. of calcium lactate there is a rise of 5–14 per cent. of calcium in the serum[20] and keeps the elevation above normal level long as for 12 hours. However the increase of the amount of calcium appears to lengthen the duration rather than to elevate the calcium content.[21] Calcium lactate is a neutral drug and has no toxic action. The influence of orally administered calcium salts on the serum calcium content have been studied by Hjört.[22] The following order is given according to the degree of its absorption power or of its solubility:

$$CaCl_2 > CaFeC_9H_9O_{10}{}^{(23)}\,(C_6H_5O_7Fe + Ca(C_3H_5O_3)_2 = C_6H_5O_7Fe\cdot Ca\,C_3H_4O_3 + C_3H_6O_3) > Ca(C_3H_5O_3)_2 > Ca\,PO_4C_3H_5(OH)_2 > CaCO_3\,.$$

(16) N. R. Blatherwick, *Arch. Int. Med.*, **14** (1914), 409.
(17) K. A. Hasselbalch, *Skand. Arch. Physiol.*, **27** (1912), 1.
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(19) J. Dadlez, *Biochem. Z.*, **171** (1926), 146.
(20) W. Bauer and M. W. Ropes, *J. Am. Med. Assoc.*, **87** (1926), 1902.
(21) B. S. Kahn and J. H. Roe, *ibid.*, **86** (1926), 1761.
(22) A. M. Hjört, *J. Biol. Chem.*, **65** (1925), 783.
(23) T. Harada, this Bulletin, **10** (1935), 82.

Animal Experiment. Experiment 1. The influence of calcium lactate on the glucose tolerance in rabbits: 200 c.c. of 37.5% glucose aqueous solution was made by aid of heat, boiled for a few minutes, and the solution was divided into two equal parts. To one part 2.5 g. of calcium lactate was dissolved while it was hot. Both solutions were boiled again for a couple of minutes under same condition and cooled down to room temperature; then the solutions were made up to 100 c.c. with sterilized distilled water.

The urine in the bladder of rabbits were first evacuated by means of a catheter, and the rabbits were allowed to fast for 24 hours and at the end of that period the urine was collected by the same means as before for the control.

Then 20 c.c. of the glucose solution was injected to one rabbit in the peritonial cavity and the glucose-calcium-lactate solution to the other. While they were kept in this condition, the blood was taken out first one half hour then one hour intervals for four hours and also at the end of twenty four hours from the veins of each rabbit.

On the urine collected at the end of twenty four hours the sugar content was determined by the method of Benedict on one hand, and the sugar content in the blood was determined by Jeghers-Myer's modification of Folin's micro-method[24] on the other. The results are tabulated in Table 2 and 3.

Experiment 2. The influence of calcium lactate on blood sugar which should be elevated by the injection of adrenalin in rabbits was also studied in same manner as Experiment 1. The injection was made as follows: One with 0.2 c.c. of adrenalin (1:1000 dilution) only as a control, and the other with the same amount of adrenalin which was followed immediately after the injection of 20 c.c. of 2.5 per cent. calcium lactate solution. The results are given in Table 4 and 5.

Experiment 3. The influence of adrenalin-glucose (Table 6-A and 7-C) of adrenalin-glucose-calcium-lactate (Table 6-B and 7-B), and of adrenalin-calcium-lactate (Table 7-A) on the glycemia and the glycosuria were studied and compared as described in Experiment 1 and 2. However in this experiment the concentration of glucose-calcium-lactate, and calcium-lactate were reduced to one half with sterilized distilled water.

(24) H. J. Jeghers and V. C. Myers, *J. Lab. Clin. Med. St. Louis*, **15** (1930), 982.

Table 1.

Condition	Volume % plasma with CO_2 reduced to 0°., 760 mm.[25]	Diastatic units[25]	24 hr. excretion of 0.1 N acid NH_3 for 60 kg.[26] (c.c.)	*p*H of plasma corresponding to CO_2 combing power[27]	Blood sugar[25] (%)	$NaHCO_3$ required to turn urine alkaline for 60 kg.[26] (g.)
Normal resting adult	75—55	15—20	0—1600	7.51—7.40	0.09—0.12	0—30
Mild diabetic acidosis	55—45	20—40	1600—4000	7.40—7.34	0.15—0.30	30—50
Moderate diabetic acidosis	45—35		4000—6000	7.34—7.28		50—65
Severe diabetic acidosis	below 35	35—75	over 6000	below 7.28	0.30—1.20	over 65

Table 2. A.

♂ Wt.: 2015 g. Glucose (15 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1063	0.1617	0.1894	0.2188	0.1457	0.1169	0.1111
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	37 c.c., 0.250%, 0.093 g.			33 c.c., 1.736%, 0.573 g.			

B.

♂ Wt.: 2125 g. Glucose (15 g.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.0968	0.1530	0.1546	0.1578	0.1457	0.1071	0.1111
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	50 c.c., 0.250%, 0.125 g.			37 c.c., 0.592%, 0.219 g.			

(25) V. C. Myers, "Pract. Chem. Anal. of Blood," St. Louis Mosby Co., (1921), 16.
(26) D. D. Van Slyke, *J. Biol. Chem.*, **33** (1918), 271.
(27) V. C. Myers and L. E. Booher, *ibid.*, **59** (1924), 699.

Table 3. A.

♂ Wt.: 2125 g. Glucose (15 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1095	0.1578	0.2205	0.2 05	0.1704	0.1119	0.0920
Urine sugar	for 24 h. before treament			24 h. after treatment			
	40 c.c., 0.250%, 0.100 g.			60 c.c., 2.000%, 1.200 g.			

B.

♂ Wt.: 1800 g. Glucose (15 g.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1034	0.1471	0.1578	0.1578	0.1500	0.1234	0.1127
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	23 c.c., 0.266%, 0.061 g.			42 c.c., 1.407%, 0.591 g.			

Table 4. A.

♂ Wt.: 2040 g. Adrenalin (0.2 c.c.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1127	0.1428	0.1851	0.1327	0.1250	0.1136	0.1127
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	115 c.c., 0.182%, 0.209 g.			60 c.c., 0.388%, 0.233 g.			

B.

♂ Wt.: 1990 g. Adrenalin (0.2 c.c.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.0938	0.1250	0.1281	0.1851	0.1472	0.1270	0.1000
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	95 c.c., 0.216%, 0.206 g.			45 c.c., 0.529%, 0.238 g.			

Table 5. A.

♂ Wt.: 2050 g. Adrenalin (0.2 c.c.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.0904	0.1327	0.1744	0.1530	0.1875	0.1630	0.1127
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	115 c.c., 0.182%, 0.209 g.			30 c.c., 1.071%, 0.321 g.			

B.

♂ Wt.: 2000 g. Adrenalin (0.2 c.c.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1027	0.1250	0.1500	0.1744	0.1401	0.1292	0.0938
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	95 c c., 0.216%, 0.206 g.			66 c.c., 0.341%, 0.225 g.			

Table 6. A.

♂ Wt.: 2280 g. Adrenalin (0.2 c.c.) and glucose (15 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1095	0.3333	0.3750	0.4545	0.5000	0.4687	0.0980
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	35 c c., 0.074%, 0.026 g.			13 c.c., 3.934%, 0.511 g.			

B.

♂ Wt.: 2300 g. Adrenalin (0.2 c.c.), glucose (15 g.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.0987	0.2586	0.2727	0.2500	0.2830	0.2885	0.0820
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	24 c.c., 0.121%, 0.029 g.			15 c.c., 2.522%, 0.378 g.			

Table 7. A.

♂ Wt.: 2240 g.: Adrenalin (0.2 c.c.) and calcium lactate (0.25 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1154	0.1667	0.1724	0.2239	0.2235	0.1875	0.1292
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	30 c.c., 0.04%, 0.012 g.			17 c.c., 4.638%, 0.789 g.			

B.

♂ Wt.: 2100 g. Adrenalin (0.2 c.c.), glucose (7.5 g.) and calcium lactate (0.25 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1154	0.3125	0.2778	0.2778	0.2459	0.2143	0.1119
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	95 c.c., 0.190%, 0.181 g.			28 c.c., 1.875%, 0.525 g.			

C.

♂ Wt.: 2135 g. Adrenalin (0.2 c.c.) and glucose (7.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1200	0.3846	0.4167	0.4167	0.3750	0.3571	0.1281
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	95 c.c., 0.130%, 0.124 g.			28 c.c., 7.200%, 2.016 g.			

Conclusion from the Results. When calcium in the form of organic salts as described above was injected with glucose (Table 2 and 3) especially with glucose-adrenalin (Table 6 and 7) into rabbits it prevented considerably the elevation of the sugar contents in both the blood and urine, which would occur when glucose alone or glucose-adrenalin without calcium was administered. However the calcium had little influence on the activity of adrenalin on the blood sugar (Table 4 and 5). Why calcium assists the combustion of the carbohydrate in the body is not clear. It is presumed that the disease of diabetes is probably attended by an imperfect calcium metabolism, thereby the combustion of carbohydrate being disturbed either directly or indirectly.

In other words the production or the activity of insulin will probably depend upon the calcium content in the blood.

Summary.

(1) The glucose tolerance on albino-rabbits was studied.

(2) The influence of calcium upon the activity of adrenalin on the blood sugar in the rabbit was experimented. It gave little change.

(3) An interesting relation between calcium and the carbohydrate metabolism of the body was observed: that is, calcium prevents considerably the elevation in both hyperglycemia and glycosuria which would occur when glucose alone or glucose-adrenalin without calcium was administered.

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ISOTOPIC SHIFT OF WATER BY DISTILLATION

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The isotopic shift of water by distillation has been observed by several authors[1] mainly at the normal boiling temperature and pressure. We have determined the shift in a heavy water sample of 2.60% deuterium content on slow vacuum distillation at several lower temperatures.

Ten milligrams water was distilled each time from 20 g. of the same sample, which was kept at a constant temperature within a few hundredths of a degree. The distillation took place within 40 to 60 minutes at a temperature difference of about half a degree. The distillate was analysed by means of a micropycnometer[2] and was compared with the original water sample as shown in the table.

(1) Lewis and Cornish, *J. Am. Chem. Soc.*, **55** (1933), 2661. Hall and Jones, *ibid.*, **56** (1934), 749. Harada and Titani, *this Bulletin*, **10** (1935), 39.

(2) Gilfillan and Polanyi, *Z. physik. Chem.*, [A], **166** (1933), 254. The original method was improved by the authors and accuracy appreciably increased.

Deuterium Content of Distillates from 2.60% Heavy Water.

Temp. of distillation (°C.)	0.05	20.42	−5.94	−6.42	0.08	21.48	21.00
Deuterium content (%)	2.42	2.49	2.39	2.38	2.38	2.46	2.47

The deuterium content of the original water sample was measured from time to time during the experiment and was proved to be constant within limits of error.

The heterogeneous equilibrium studied here might be expressed by the following formula:

$$H_2O_{vap} + HDO_{liq}\text{-}xH_2O_{liq} = HDO_{vap} + H_2O_{liq}\text{-}xH_2O_{liq},$$

where xH_2O_{liq} denotes environments of the molecule in the liquid state. H_2O_{liq}-xH_2O_{liq} may be a polymer. D_2O concentration was neglected here and environments both of H_2O and of HDO molecules were assumed to be the same, because of the low deuterium content. The measured shift of the distillate gives directly the equilibrium constant of the reaction, $RT \ln \frac{p_{HDO}}{p_{H_2O}} + \text{const.}$ The heat toning of the reaction or the difference between partial molar heat of vaporisation for HDO and H_2O was calculated from the temperature variation of the equilibrium constant as -215 ± 24 calories.

The heat toning of the reaction might also be correlated with spectroscopic data. We assumed that the rotational energies are fully excited at the temperatures, that the rotational degree of freedom in liquid phase is exactly the same for HDO_{liq} and for H_2O_{liq} and that vibrational energies are at their lowest quantum states both for single molecule and for polymers in the liquid. The heat toning Q is then given by the difference of zero point energies of aggregates,

$$Q = \frac{1}{2}\sum h\nu_{H_2O_{vap}} + \frac{1}{2}\sum h\nu_{HDO_{liq}\text{-}xH_2O_{liq}} - \frac{1}{2}\sum h\nu_{HDO_{vap}} - \frac{1}{2}\sum h\nu_{H_2O_{liq}\text{-}xH_2O_{liq}},$$

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where νs means fundamental vibrational frequencies. Fundamental frequencies were given for H_2O_{vap} and HDO_{vap} by Bartholomé and

Clusius,[3] and by Wood,[4] and for liquid by Ellis and Sorge.[5] Ellis and Sorge assume the fourth fundamental frequency ω^* for polymerisation and give 2130 cm.$^{-1}$ for H_2O and 1640 cm.$^{-1}$ for D_2O. Should Q by the above expression give the measured heat toning taking ω^* into account, lacking data of ω^* for HDO would be 1740 cm.$^{-1}$, which lies between those for H_2O and for D_2O. The expression for Q would give positive value +300 cals. without taking ω^* into consideration, in contrary to the measured endothermicity of the reaction.

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(3) Bartholomé and Clusius, *Z. Elektrochem.*, **40** (1934), 529.
(4) Wood, *Phys. Rev.*, **45** (1934), 732.
(5) Ellis and Sorge, *J. Chem. Phys.*, **2** (1934), 558.